**DATA EVALUATION RECORD**

129099

1. **CHEMICAL**: NTN 33893
   Shaughnessy No. ~~129059~~

2. **TEST MATERIAL**: Technical NTN 33893, 96.2%.

3. **STUDY TYPE**: Acute Toxicity Test for Estuarine and Marine Organisms, Mysids (Mysidopsis bahia).

4. **CITATION**: Ward, G.S. 1990. "NTN 33893 Technical: Acute Toxicity to the Mysid, (Mysidopsis bahia)". Toxikon Environmental Sciences, 106 Coastal Way, Jupiter Florida 33477. Laboratory Report No. J9008023b,f. Submitted by Mobay Corporation, Research and Development Department, P.O. Box 4913, Kansas City Missouri 64120. US EPA MRID No. 420553-19.

5. **REVIEWED BY**:

   Dana Lateulere, Biologist
   Ecological Effects Branch
   Environmental Fate and
   Effects Division

   **Signature:** Dana Lateulere
   **Date:** 3/27/92

6. **APPROVED BY**:

   Ann Stavola, Section Head, 5
   Ecological Effects Branch
   Environmental Fate and
   Effects Division

   **Signature:** Ann Stavola
   **Date:** 3/27/92

7. **CONCLUSIONS**: This study is scientifically sound but does not fulfill the guideline requirement of Acute Toxicity Testing for Estuarine and Marine Organisms, a NOEC was not achieved. Two tests were performed; test #1 did not test concentrations low enough to achieve a NOEC, test #2 had 15% mortality in the control. Test #1 is supplemental, test #2 is invalid. Based on the results of test #1, the LC50 is 37.7 ug a.i./L, with 95% confidence limits of 2.34 and 6.06 ug/L.

8. **RECOMMENDATIONS**: Repeat the test with lower concentrations in order to achieve a NOEC.

9. **BACKGROUND**: This study was submitted as part of registration and EUP requirements.

File

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

**MEMORANDUM**

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

**SUBJECT:** NTN 33893 Ecological Effects Data, response to Miles Inc.'s request to upgrade four aquatic studies:
**#72-3 c**, MRID No. 420553-19 classified as supplemental.
**#72-4 a**, MRID No. 420553-20 classified as invalid.
**#72-4 b-freshwater**, MRID No. 420553-21 classified as supplemental.
**#72-4 b-estuarine/marine**, MRID No. 420553-22, classified invalid.

**FROM:** Douglas Urban, Acting Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

Douglas J. Urban 8/25/92

AUG 25 1992

**TO:** Dennis Edwards, PM 19
Registration Division (H7505C)

EEB has reviewed Miles Inc.'s requests, submissions and scientific conclusions regarding the classification of the following aquatic studies. The response to the request for the reclassification of each study to "core" is as follows (per case study):

**1. Mysid Acute (72-3) - MRID No. 420553-19:** Two studies were performed and submitted for this requirement (herein referred to as Part 1 and Part 2); Part 1 was classified as supplemental based on the lack of a discernable NOEC, Part 2 was classified as invalid based on high control mortality. Miles Inc. states: "the two together should satisfy the guideline requirement, a NOEC is not a guideline requirement for an acute test, the control mortality is irrelevant in that Part 2 was only conducted to supplement a NOEC".

Part 2 is invalid and will not be upgraded. Part 1 will be upgraded to "core". The statement from Anne Barton (made on July 13, 1992) that "a no mortality level for acute studies should not be a requirement, but it would be preferable to have this information" clearly addresses any confusion in the branch which stems from the EPA SEP for Acute Toxicity Tests for Aquatic Invertebrates (1985) which states, "results from a valid study **should** provide a zero mortality level and a slope of the dose-mortality response line." This clarification is noted, this situation will not be repeated.

**2. Daphnia Life Cycle (72-4) - MRID No. 420553-21:** The study was classified as supplemental based on the lack of dry weight data. Guidance in this area needs to be improved. The EPA publications indicate that length measurements are adequate to analyze effects

on growth. However, ASTM, 1987, determined that weight is a better growth indicator than length. An amendment to the SEP was drafted in September 1990 and indicated that dry weight and length measurements are needed. Laboratories were notified, including ABC Labs, of the amendments. However, these changes have not been incorporated formally into EPA publications and therefore, cannot be used as a means to reject a study. The daphnid life cycle is upgraded to "core".

**3. Trout Early Life Stage (72-4) - MRID No. 420553-20:** The study was classified as invalid based on solvent control results which showed a positive effect on fish growth, and a NOEC was not determined for survival or percent swim-up. Miles disagrees with both points and wishes an upgrade to "core" be granted.

Al Jarvinen, EPA ERL Duluth, MN agrees with EEB that the case of the solvent control positively effecting fish growth and survival is unusual but not obsolete. However, after reviewing the results and methods of the study with A. Jarvinen, it was concluded that the results are useable. Statistical analysis will be performed with the data from the solvent control (not the dilution water control as reported) when the raw data is submitted, which Miles' has agreed to submit. The study will be re-classified as "supplemental" with the option of repairability to "core" upon data submission and review.

**4. Mysid Life Cycle (72-4) - MRID No. 420553-22:** This study was classified as invalid based on deviations from protocol in the test design. EEB has reviewed Mile's request for an upgrade to "core". Review of alternate protocol designs was performed and EEB concurs that the design used was adequate. The Mysid Life Cycle will be upgraded to "core". The MATC that will be used in risk analysis is 230 ng/L, as agreed upon by Miles Inc. in their submission.

EEB feels this memo should 'clear-up' the concerns Miles Inc. had for the outcome of these studies and thus, the meeting planned for Aug. 27 at Crystal Station II, is no longer necessary.

**10. DISCUSSION OF INDIVIDUAL TESTS:** N/A.

**11. MATERIALS AND METHODS:**

**A. Test Animals:** Post-larval mysids were obtained from mysid cultures maintained at Toxikon Environmental Sciences, Jupiter, FL. Upon collection, post-larval mysids were fed live brine shrimp nauplii hatched daily from cysts obtained from Glen Burnie MD. No diseases were observed and no disease treatments were administered to either the adult culture population or the post-larvae.

**B. Test System:** The dilution water was natural filtered saltwater adjusted to a salinity of approximately 20 ppt. The dilution water was vigorously aerated prior to use. The definitive exposure was conducted under flow-through conditions in a modified proportional vacuum-siphon diluter system. The test system was volumetrically calibrated to provide a test concentration series with a 60 percent dilution and equal solvent concentration in all test concentrations. The test solution was prepared from stock solution in a mixing chamber. The test solution was diluted in the diluter system to provide the four lower test concentrations. Both dilution water control and solvent control(DMF) were maintained concurrently with the five test solution.

A test solution volume of approximately 1400 ml was delivered to each test chamber during every cycle. Test chambers were 24 liter glass tanks equipped with automatic glass siphons. The siphons were positioned to provide a maximum depth of 13 cm and volume of 15 liters. The diluter cycled at a rate of 3.8 cycles per hour providing 8.5 volume additions every 24 hours.

Mysids were impartially added, by twos, to 30-ml plastic cups until 20 mysids were distributed to each. The mysids were then transferred to screened retention chambers positioned in each test chamber; one group of 20 to each treatment with no replications. The test chambers were randomly positioned in a water bath under fluorescent lighting regulated to an overall photoperiod of 16 hours light and 8 hours dark with a 30 minute transition period to simulate dawn and dusk. Light intensity ranged between 350 and 475 lux.

**C. Dosage:** Test #1, mean measured concentrations were: 0, 0, 31.9, 58.4, 93.7, 146.0, 249.0 ug a.i./L. Test #2,

mean measured concentrations: 0, 0, 8.42, 13.3, 22.9, 37.2, 63.4, ug a.i./L.

D. **Design:** Test water quality was monitored each day during the test. Water temperature in the dilution water control was continuously monitored by a min/max thermometer and spot checked with a hand held digital thermometer daily. Salinity of the dilution water was measured with a refractor in the control test chamber daily during the test. Dissolved oxygen concentrations and pH were measured daily in all test solutions with surviving mysids.

E. **Statistics:** The LC50 values were estimated by a computer program using the following statistical methods: moving average angle, probit, logit, and non-linear interpolation. Confidence limits for LC50 values determined by non-linear interpolation were calculated by binomial probability.

12. **REPORTED RESULTS:** Mortality of mysids exposed for 96 hours to NTN-33893 technical ranged from 0 percent in a mean measured test concentration of 31.9 ug/L to 100 percent in a mean measured test concentrations ≥93.7 ug/L. There was no mortality in the dilution water and solvent controls. After 96 hours of exposure, one mysid in 31.9 and 58.4 ug/L was lethargic and two mysids in 58.4 ug/L exhibited some loss of equilibrium. The 96-hour LC50 based on mean measured NTN-33893 concentrations was 37.7 ug/L with 95% confidence limits of 26.7 to 46.4 ug/L. The slope of the toxicity curve was 4.2. The NOEC was not determine, based upon the presence of mortality and sublethal effects in all test concentrations.

The test temperature during the 96 hour exposure ranged from 19.9 to 22.7°C. The salinity of the dilution water remained between 20 and 22 ppt during the test. Dissolved oxygen concentrations remained ≥4.4 mg/L in all test containers during the test. pH values ranged from 8.4 to 8.6 in all test containers during the test.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Quality Assurance Inspection was conducted for compliance verification by the Quality Assurance Unit. It was also stated that this study was conducted in compliance with the Good Laboratory Practice Standards, 40 CFR Part 160.

**14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

**A. Test Procedure:** The test procedures were in accordance with Subdivision E, and SEP guidelines except for the following deviations:

- Test temperature should be 22°C and not deviate more than 1°C during the test; the temperature ranged from 19.9 to 22.7°C.

- A No-effect concentration was not determined.

**B. Statistical Analysis:** Toxanol was used to determine the LC50 and its 95% confidence interval. The method utilized for this test was the probit method.

**C. Discussion/Results:** The 96 hour LC50 was 37.2 ug a.i./L as determined by the Probit method. The 95% confidence interval is 26.7 - 46.4 ug/L. The NOEC was determined to be less than 31.90 ug/L, the lowest concentration tested, based on mortality at this level; therefore, a NOEC was not determined. Test #1 is classified as supplemental; lower test concentrations should be used to determine a NOEC. Test #2 is classified as invalid due to the control mortality; results from this study cannot be used.

**D. Adequacy of the Study:**

**(1) Classification:** Supplemental.

**(2) Rationale:** Failure to achieve a NOEC.

**(3) Repairability:** No. The study could only be repeated utilizing lower test concentrations.

Table 2. Mortality of Mysid, Mysidopsis bahia, Exposed to NTN-33893 under Flow-Through Test Conditions (Test Conducted September 10 - 14, 1990)

| Mean Measured Concentration (µg/L; ppb) | Cumulative Number Dead (Percent Mortality) 24 Hour | 48 Hour | 72 Hour | 96 Hour |
|---|---|---|---|---|
| Control | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Sol Ctl | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 31.9 | 0 (0) | 0 (0) | 2 (10) | 9 (45)[e] |
| 58.4 | 0 (0) | 1 (5) | 8 (40) | 13 (65)[f] |
| 93.7 | 1 (5)[a] | 19 (95) | 19 (95)[c] | 20 (100) |
| 146 | 3 (15)[b] | 18 (90)[d] | 20 (100) | 20 (100) |
| 249 | 7 (35)[c] | 20 (100) | 20 (100) | 20 (100) |

[a] One mysid exhibited a partial loss of equilibrium.

[b] Three mysids were lethargic.

[c] Eight mysids were lethargic.

[d] Two mysids remaining were lethargic.

[e] One mysid was lethargic.

[f] One mysid was lethargic and two exhibited a partial loss of equilibrium.

Table 4. Daily Temperature Range During a 96-Hour Flow-Through Exposure of Mysid, Mysidopsis bahia, to NTN-33893 (Test Conducted September 10 - 14, 1990)

| Exposure Period (Hours) | Temperature* (°C) |
|---|---|
| 0 - 24 | 19.9 - 22.3 |
| 24 - 48 | 19.9 - 22.5 |
| 48 - 72 | 21.1 - 22.7 |
| 72 - 96 | 20.9 - 22.7 |

* Daily temperature ranges reported are the minimum and maximum hourly temperatures recorded by the data logger.

Table 7. Measured Concentrations of NTN-33893 During a Second 96-Hour Exposure of Mysid, Mysidopsis bahia, Under Flow-Through Conditions (Test Conducted September 26 - 30, 1990)

| Nominal Concentration (µg/L; ppb) | *Measured Concentration (µg/L) 0 Hr | 96 Hr | Mean (±SD) | Percent of Nominal |
|---|---|---|---|---|
| Control | ND | ND | ---- | ---- |
| Sol Ctl | ND | ND | ---- | ---- |
| 8 | 8.68 | 8.17 | 8.42 (0.36) | 105 |
| 13 | 13.7 | 12.9 | 13.3 (0.52) | 102 |
| 22 | 23.5 | 22.2 | 22.9 (0.96) | 104 |
| 36 | 37.6 | 36.9 | 37.2 (0.47) | 103 |
| 60 | 63.1 | 63.7 | 63.4 (0.45) | 106 |
| **MATRIX SPIKE RECOVERY DATA** | | | | |
| MS-1 | 21.5 | 20.7 | 20.8 (0.50) | 104 |
| MS-2 | 20.9 | 20.3 | | |

SD = Standard Deviation.

ND = Not detected; the limit of detection for the method was 0.5 µg/L.

MS = Matrix spike. Matrix spikes consisted of test substance in dilution water and were duplicated. The spike concentration was 20 µg/L.

* Day -1 measured test concentrations for nominal concentrations of 8, 22 and 60 µg/L were 113, 133 and 101 µg/L, respectively.

Table 8. Mortality of Mysid, Mysidopsis bahia, Exposed to NTN-33893 under Flow-Through Test Conditions During a Second 96-Hour Exposure (Test Conducted September 26 - 30, 1990)

| Mean Measured Concentration (µg/L; ppb) | Cumulative Number Dead (Percent Mortality) | | | |
|---|---|---|---|---|
| | 24 Hour | 48 Hour | 72 Hour | 96 Hour |
| Control | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Sol Ctl | 0 (0) | 0 (0) | 3 (15)[e] | 3 (15) |
| 8.42 | 0 (0) | 0 (0) | 0 (0) | 1 (5)[e] |
| 13.3 | 0 (0)[a] | 0 (0) | 0 (0) | 0 (0) |
| 22.9 | 2 (10)[a] | 2 (10)[a] | 2 (10) | 2 (10) |
| 37.2 | 11 (55)[b] | 11 (55)[d] | 12 (60) | 12 (60) |
| 63.4 | 17 (85)[c] | 20 (100) | 20 (100) | 20 (100) |

[a] One mysid exhibited a partial loss of equilibrium.

[b] Two mysids exhibited a partial loss of equilibrium.

[c] All surviving mysids exhibited a partial loss of equilibrium.

[d] One mysid exhibited a partial loss of equilibrium and one was lethargic.

[e] Mysids recorded as dead were not found; presumably cannibalized.

LATEULERE NTN 33893 MYSID LC50

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 249 | 20 | 20 | 100 | 9.536742E-05 |
| 146 | 20 | 20 | 100 | 9.536742E-05 |
| 93.7 | 20 | 20 | 100 | 9.536742E-05 |
| 58.4 | 20 | 13 | 65 | 13.1588 |
| 31.9 | 20 | 9 | 45 | 41.19014 |

THE BINOMIAL TEST SHOWS THAT 0 AND 93.7 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 37.0532

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 1 | 2.520492 | 37.05319 | 0 | +INFINITY |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 5 | .1961654 | 1 | .2791287 |

SLOPE = 4.205869
95 PERCENT CONFIDENCE LIMITS = 2.343066 AND 6.068671

LC50 = 37.72011
95 PERCENT CONFIDENCE LIMITS = 26.73207 AND 46.35239

LC10 = 18.81976
95 PERCENT CONFIDENCE LIMITS = 8.258386 AND 26.60193

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NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.
THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

LATEULERE NTN 33893 MYSID ACUTE LC50

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 63.4 | 20 | 20 | 100 | 9.536742E-05 |
| 37.2 | 20 | 12 | 60.00001 | 25.17223 |
| 22.9 | 20 | 2 | 10 | 2.012253E-02 |
| 13.3 | 20 | 0 | 0 | 9.536742E-05 |
| 8.42 | 20 | 1 | 5 | 2.002716E-03 |

THE BINOMIAL TEST SHOWS THAT 22.9 AND 63.4 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 34.07613

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 3 | 5.351207E-02 | 33.32873 | 29.15649 | 38.6728 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 5 | 3.884588 | 12.4405 | 0 |

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 5.106125
95 PERCENT CONFIDENCE LIMITS =-4.957721 AND 15.16997

LC50 = 32.38785
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 18.26675
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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